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EFFECTS OF PULSE CHLORINATION AND TEMPERATURE ON RAINBOW SMELT EGGS AND LARVAE

Report No 82-386-K

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ABSTRACT

Rainbow smelt eggs were pulse chlorinated for 2 hrs/day (0 to 650 ppb TRC) at constant temperatures from 7°C to 20°C. Temperature affected total hatch, but chlorine did not. Viable hatch was affected by both temperature and chlorine. Deformity and larval mortality were the main causes of difference between total hatch and viable hatch results. Larval deformity increased sharply at high temperatures but was unaffected by chlorination. Larval mortality increased rapidly above 200 ppb TRC, reaching 50% near 400 ppb, but was not significantly affected by temperature. Smelt larvae are much more chlorine-sensitive than eggs, but Ontario Hydro thermal discharge concentrations (100 ppb TRC maximum) should be well below toxic levels. Due to turbulent discharge flows and intermittent chlorination, few larvae should experience maximum chlorine concentrations.

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To Mr. D.K.A. Gillies
Manager
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EFFECTS OF PULSE CHLORINATION AND TEMPERATURE ON RAINBOW SMELT EGGS AND LARVAE

INTRODUCTION

Field studies have indicated that rainbow smelt (Osmerus mordax) have spawned in the thermal discharges of Lakeview GS, Lennox GS and Nanticoke GS (Ontario Hydro Research Division Reports 80-123-K, 78-28-K and 82-59-K). Great Lakes rainbow smelt are primarily stream spawners, ascending tributary streams to spawn during the April to May period. However, some shore spawning probably occurs on wave-washed shoals (Scott and Crossman, 1973). Smelt may be attracted to both the current and the elevated temperatures created by thermal discharge plumes. Although smelt spawning has been observed only at three Ontario Hydro thermal generating stations, the potential exists for spawning at all Great Lakes thermal generating stations which use once-through cooling.

The present study was undertaken to assess the potential effects of condenser cooling water chlorination on smelt eggs spawned in thermal discharge areas. There was no available data on responses of rainbow smelt eggs to chlorine. There was only minimal data in the published literature on eggs or early larvae of other freshwater fishes subjected to repeated (pulse) chlorination typical of power plant condenser cooling water discharges (see review by Opresko, 1980). The present study was initiated and funded by the Environmental Protection Department of the Thermal Generation Division (Memo: Gillies to Clark, November 25, 1980).

Ontario Hydro thermal stations follow a variety of chlorination schedules, depending upon local water quality and season.[1] The Thermal Generation Division has undertaken optimization studies since 1978 to reduce chlorine application to the minimum required for control of condenser biofouling (Butler et al, 1981). Of those generating stations where regular chlorination is required, daily dosage is usually employed during the April to May period. The units of a generating station are chlorinated in sequence, resulting in a maximum chlorine discharge of 100 ppb total residual chlorine (TRC) or less for approximately two hours/day.

The present study was designed to determine the effects (if any) of repeated daily chlorination at TRC levels and temperatures typical of thermal discharge values. In addition, higher TRC levels were included to assure that measurable toxicity would occur.

[1]The following Ontario Hydro Thermal Generating Stations chlorinate during the late spring period: Lakeview, Hearn, Lambton, Keith, Thunder Bay.

METHODS

1. Experimental Design

The present study was designed to meet two primary objectives:

a) Determine the effects (if any) of repeated daily chlorination (2 hrs/day) and temperature on smelt eggs exposed to chlorine levels typical of maximal discharge values (100 ppb TRC) throughout the incubation period.

b) Determine toxic levels (LC50) for repeated pulse chlorination through exposure of smelt eggs to TRC levels well in excess of discharge concentrations, to define the safety margin (if any) of present Ontario Hydro chlorination practises.

Experiments were arranged in a geometric series of chlorine concentrations to allow both of the previous objectives to be met. A wide range of temperatures, typical of those likely in a thermal discharge, were included on the experimental design. Our previous thermal studies indicated that the limits for successful smelt egg incubation range from below 5°C to approximately 23°C (Ontario Hydro Research Division Report 78-343-K). For the present study, test temperatures of 7, 12, 17 and 22°C were chosen. However, as excessive mortalities were observed at the highest temperature early in the experiment, the 22°C treatments were replaced with a new group of eggs incubated at 20°C. A fractional factorial experimental design was employed (Figure 1) which allowed testing of six chlorine levels plus controls at four temperatures with only 16 data points. This design was chosen as it allowed a wide range of chlorine levels to be tested, yet limited the number of chlorine determinations required on each test day. Two concurrent replicates of 300 to 500 eggs were run at each of the temperature/chlorine combinations shown in Figure 1. Nominal chlorine concentrations used in this study were 0, 50, 130, 230, 350, 490 and 650 ppb (µg/L) total residual chlorine.

2. Experimental Apparatus and Procedures

The egg incubation apparatus consisted of four temperature-controlled head tanks, each supplying dilution water by gravity feed to eight flow-through incubation chambers (Figure 2). Smelt eggs spawned onto frosted glass plates were placed in Nitex screened (363 µ) containers. The containers fit into the incubation chambers in a manner which assured good water flow past the eggs. Water replacement times in the incubation chambers approximated 5 minutes.

Chlorine was introduced as a concentrated NaOCl solution in the water supply lines upstream of the incubation chambers. Dilution water (dechlorinated Toronto tap water from Lake Ontario) was metered by needle valves and flowmeters (Figure 2). Each treatment was pulse chlorinated for 2 hours/day from fertilization until complete hatch occurred (8 days @ 20°C; 33 days @ 7°C).

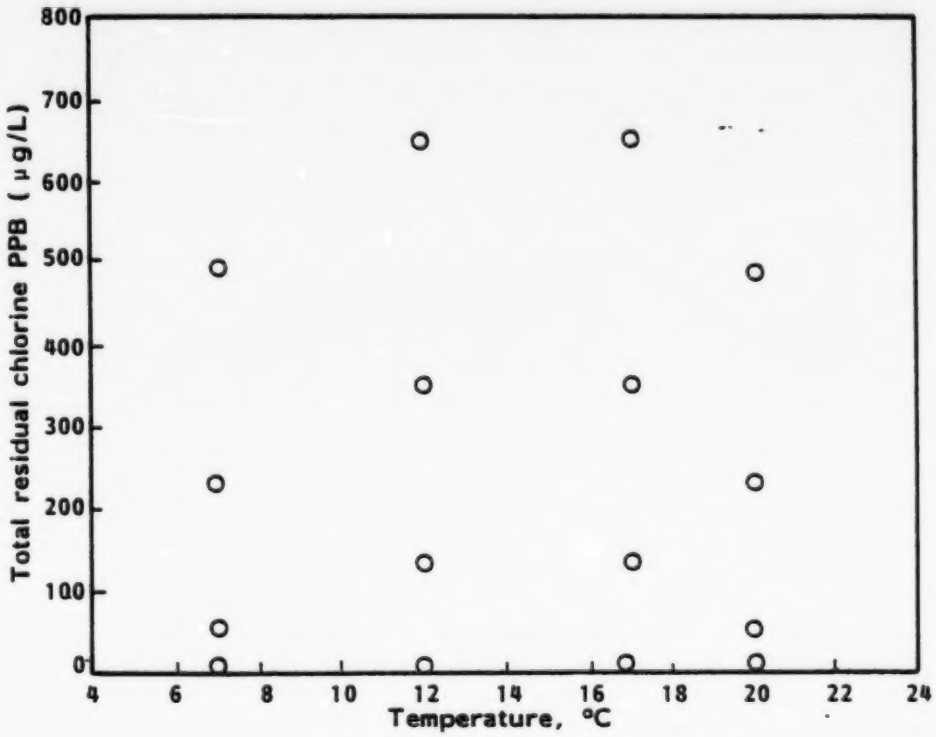

FIGURE 1

EXPERIMENTAL DESIGN FOR CHLORINE/TEMPERATURE BIOASSAY OF SMELT EGGS. 2 REPLICATES OF 300-500 EGGS WERE RUN AT EACH CHLORINE/TEMPERATURE COMBINATION

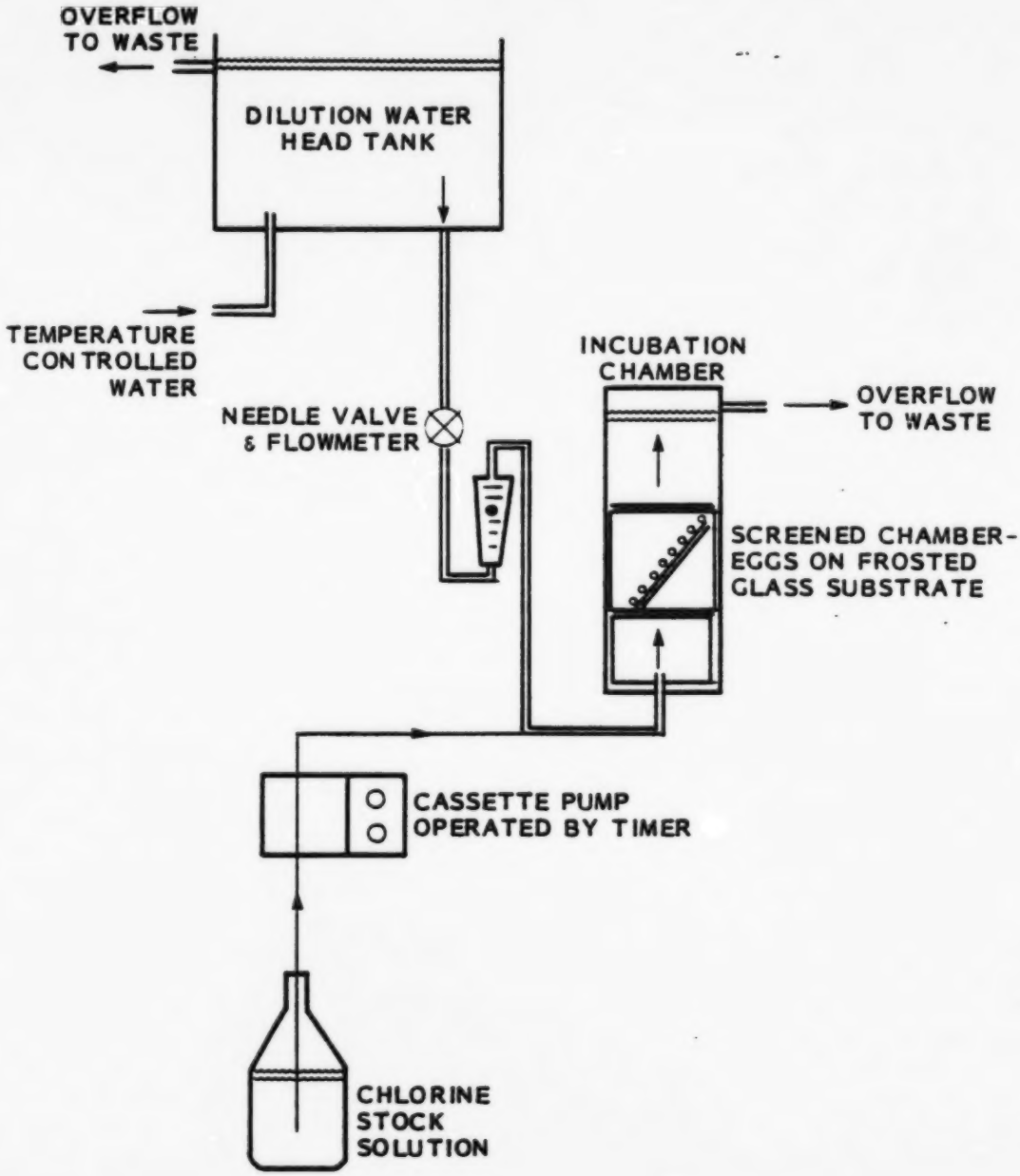

FIGURE 2

SCHEMATIC DIAGRAM OF APPARATUS
FOR PULSE CHLORINATION OF SMELT EGGS

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In order to allow time for daily chlorine measurements in each treatment, half the incubation chambers were chlorinated in one two-hour period, followed by the remainder in a second two-hour period. The peristaltic pumps which delivered the chlorine solution were operated by a timer. Due to rapid flow rates through the incubators, wash-in and wash-out periods during each chlorination pulse were quite short (approximately 5 minutes).

Adult smelt were collected from Lake Ontario during their spawning run in mid-April of 1981. In the laboratory, eggs were dry-spawned onto frosted glass substrates, rinsed, and placed in the incubation apparatus at desired test temperatures within a few minutes of fertilization. Initial egg numbers on each glass substrate were determined from macro-photographs taken shortly after fertilization. The high-contrast black and white negatives were projected on a microfilm reader to allow accurate counts of egg numbers in each incubation chamber. The procedure provided a permanent record of initial egg numbers and minimized handling of the fragile newly fertilized eggs.

Pulse chlorination began the following morning, within 18 hours of fertilization. Egg developmental rate and survival were monitored throughout the experiment until hatch was completed in all treatments. Dead eggs were counted and removed daily to minimize growth of fungus on the eggs. Daily mortality counts allowed determination of any temporal changes in embryo sensitivity to combined chlorine and temperature stress.

3. Chlorine Analysis

Total residual chlorine (TRC) levels in each treatment were determined during each daily dosing period, using amperometric titration methods developed by Analytic Services and Central Thermal Services (Ontario Hydro Research Division Report No 75-21-H). In this method, samples were buffered to pH 4.7, KI was added and the sample was then titrated with phenyl arsine oxide to determine total residual chlorine. As used, the method was capable of measuring TRC concentrations from 2 ppb to 1000 ppb, with an expected precision of ±10% at 20 ppb (O.Herrmann, Analytic Services, personal communication).

4. Data Analysis

Results of the fractional factorial design were used to fit response surface models describing the combined effects of residual chlorine and temperature on smelt egg survival. The analysis was performed by methods outlined by Lindsey et al (1970) using the response surface program of Lindsey and Sandnes (MS 1972). Adaptation of this program to Hydro's UNIVAC computer was described in Ontario Hydro Research Division Report No 75-484-K. The general form of the models fitted was:

$$Y^{\gamma} = b_0 x_0 + b_1 x_1^{\alpha 1} + b_2 x_2^{\alpha 2} + b_{11} x_1^{2\alpha 1} + b_{22} x_2^{2\alpha 2} + b_{12} x_1^{\alpha 1} x_2^{\alpha 2}$$

where:

Y = calculated response (percent viable hatch, percent deformed larvae, percent mortality of newly hatched fry)

$b_0 x_0$ = intercept value (where $x_1 = x_2 = 0$)

x_1 = incubation temperature °C

x_2 = total residual chlorine µg/L

$\gamma, \alpha_1, \alpha_2$ = transformations of the dependent and independent variables selected by an iterative procedure converging on those transformations yielding a minimum residual variance

The fitted models were evaluated through Analysis of Variance and examination of residuals (differences between observed data values and values predicted by the models). Results were expressed as contour plots, graphically displaying the interaction of residual chlorine and temperature on egg survival and mortality of newly hatched fry.

5. Experiments With Lakeview GS Cooling Water

Dilution water used in the present experiments (Toronto tap water) had been subjected to considerable treatment prior to its use in these bioassays. In the water filtration plant, sand filtration would be followed by chlorination. The water was then dechlorinated in the laboratory by filtration through activated charcoal, a process which would also remove any fine particulate matter.

In discussions with CTS staff members, it was suggested that bioassay tests with raw cooling water from generating stations such as Lakeview might yield different results due to higher concentrations of particulate matter (organic and inorganic debris) and a variety of trace contaminants contained in nearshore Lake Ontario waters. Accordingly, a limited test series was conducted (at 17°C) using raw Lakeview GS condenser cooling water as a dilution source. Nominal chlorine concentrations of 0, 350 and 650 ppb TRC were tested (for 2 hours/day) using two replicates of 300 to 500 eggs for each treatment. The water was obtained from the Lakeview GS thermal discharge during non-chlorination periods. The incubation chambers using Lakeview water were set up as a recirculating system. The water was aerated and temperature controlled in a 1200 liter reservoir. Water from this reservoir was circulated through the incubators and returned during non-chlorination periods. During chlorination, overflow from the egg incubators was routed to waste by a solenoid valve to prevent contamination of the reservoir. Due to losses of the dilution water during these periods, it was necessary to replenish the reservoir every four days during the duration of this experiment (12 day period).

Results of the experiment with Lakeview GS dilution water were compared with response surface models derived from the main experiment to determine whether substantial differences in response might be expected in typical generating station discharge waters.

RESULTS

1. Temporal Patterns of Mortality

Mortality during embryonic development followed a distinct temporal pattern, being highest in the early developmental stages. Similar results were observed in previous thermal studies of smelt eggs (Ontario Hydro Research Division Report No 78-343-K). Peak mortality occurred during epiboly, declining substantially after closure of the blastopore and formation of a free tail-bud (developmental stage 8, Appendix 1). Separate survival models were fitted to the data, considering the early mortality peak (Developmental Block 1, fertilization to stage 8) and the remainder of the developmental period (Block 2, stage 8 to completion of hatch). In addition, a summary model was fitted, describing effects of temperature and pulse chlorination on survival throughout the entire incubation period.

2. Early Embryonic Survival

Survival data for the early embryonic period (fertilization to stage 8) are listed in Appendix 2A. A second order polynomal model was fitted to the results (Appendix 2B). A contour plot of this model (Figure 3) suggests that best survival occurred over the 10°C to 14°C range, declining somewhat at higher temperatures. There was little change in survival as chlorine concentrations increased from 0 ppb to about 500 ppb. At the highest chlorine concentrations (650 ppb) the model suggests a slight increase in survival. The high survival suggests that at the concentrations tested, pulse chlorination may aid embryo survival, possibly by decreasing fungal infections, which are quite common in developing smelt eggs. Presumably, the chorion surrounding the developing embryo provides sufficient protection from chlorine at the concentrations tested.

It should be noted that the trends shown in Figure 3 have not been well defined (Appendix 2B). This appears to be the result of high variance between a few replicate chambers (Appendix 2A). Attempts to improve the model through transformation of variables were not successful. Thus, it must be concluded that the range of temperatures (7°C to 20°C) and TRC concentrations (0 to 650 ppb) was not sufficient to clearly define effects during early development, given the level of effort (two replicates) and the variability inherent in the data. Inclusion of higher temperatures (eg, 22°C) would have yielded significant thermal effects, but the resultant high mortality would have precluded estimates of chlorine effects on later developmental stages.

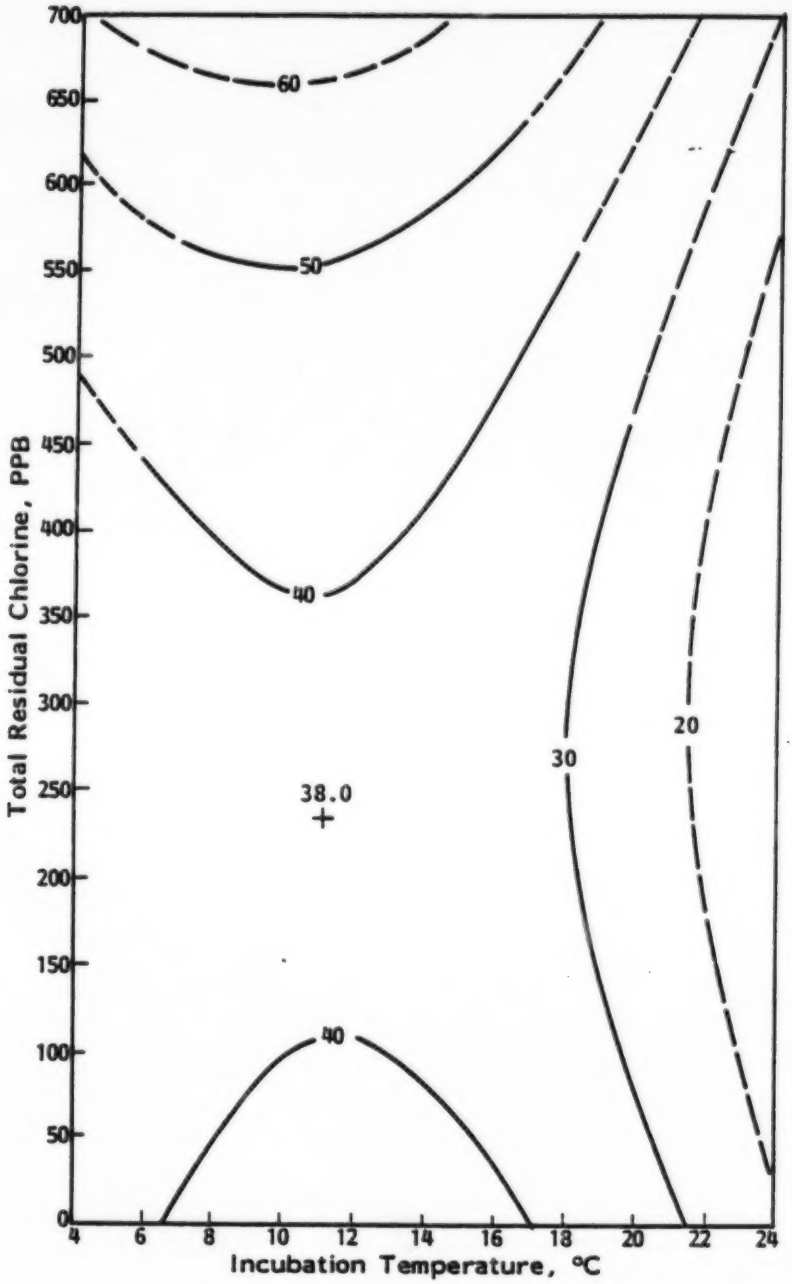

FIGURE 3

EFFECTS OF CHLORINE AND TEMPERATURE ON SMELT EGGS IN EARLY DEVELOPMENT. CONTOURS IN PERCENTAGE SURVIVAL - FERTILIZATION TO STAGE 8 (BLOCK 1)

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3. Embryonic Survival During Later Development

Survival data for the period from developmental stage 8 to completion of hatch (Block 2) are listed in Appendix 3A. Analysis of the response surface model fitted to these data indicates a significant effect of temperature on embryo survival, but no significant effect of chlorination (Appendix 3B).

The contour plot of the model (Figure 4) clearly indicates that survival during the later developmental stages (Block 2) was much higher than during early (Block 1) development. Best survival (>80%) was obtained over the 8°C to 15°C range. At higher temperatures, survival declined substantially, reaching the 50% to 60% range at 20°C. The model suggests a slight interaction of chlorine and temperature, as maintenance of a given survival at high chlorine levels is coupled with slightly decreased temperature. However, as indicated by the Analysis of Variance (Appendix 3B), this effect was not statistically significant.

4. Summary Survival Model (Total Hatch)

Results for the entire incubation period (fertilization to complete hatch) were used to fit a summary survival model (Appendix 4). As indicated by the Analysis of Variance, temperature affected the survival (percentage total hatch) of smelt eggs, but pulse chlorination did not. The contour plot (Figure 5) is quite similar to that obtained from the Block 1 data, indicating the importance of early mortality in defining overall survival through development. Best survival (30% to 40%) is indicated for the 8°C to 16°C range, declining with increased temperature to less than 20% at 20°C.

5. Viable Hatch

In assessing potential effects of pulse chlorination, one must consider not only survival of eggs throughout development, but also the numbers of live, healthy larvae produced, that is, the viable hatch. Viable hatch data for the experiment are listed in Appendix 5A. As indicated by the Analysis of Variance for the fitted model (Appendix 5B), both temperature and chlorine concentration significantly affected viable hatch. A contour plot of the model (Figure 6) indicates that the best viable hatch (>30%) occurred over the 7°C to 12°C range, with TRC concentrations less than 100 ppb. Both higher temperatures and higher chlorine concentrations resulted in a progressive decline in viable hatch. At TRC concentrations in the 500 to 600 ppb range, viable hatch was reduced to 5% under favourable temperatures, while extremely warm temperatures (20°C) resulted in approximately 5% viable hatch even in absence of chlorine. At a residual chlorine level of 100 ppb (maximum expected in station discharges) viable hatch of smelt eggs would be only marginally lower than that expected in non-chlorinated water. Substantial decrease in viable hatch becomes apparent at TRC levels above 200 ppb, with survival levels at 400 ppb approximating 50% of those expected in absence of chlorine.

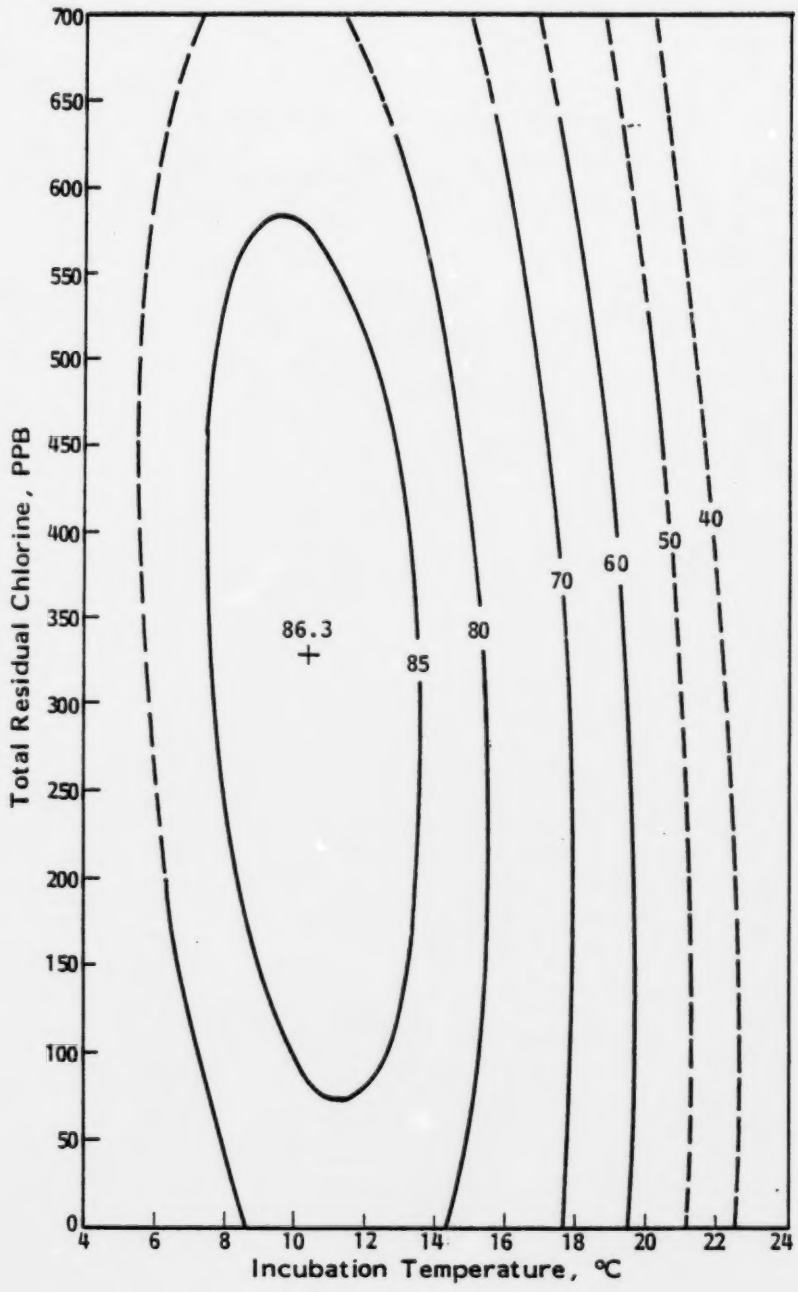

FIGURE 4

EFFECTS OF CHLORINE AND TEMPERATURE ON SMELT EGGS IN LATER DEVELOPMENT - STAGE 8 TO HATCH (BLOCK 2). CONTOURS IN PERCENTAGE SURVIVAL

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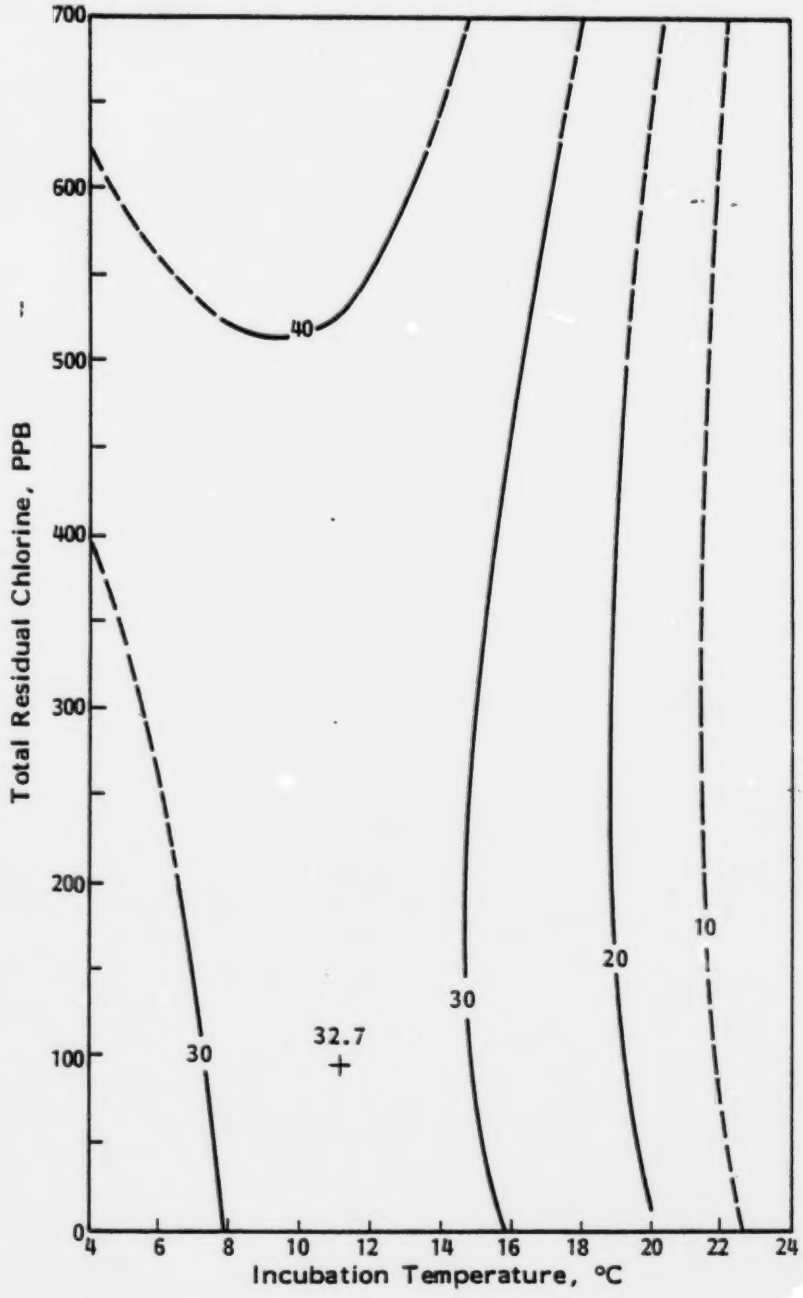

FIGURE 5

EFFECTS OF CHLORINE AND TEMPERATURE ON TOTAL HATCH OF SMELT EGGS. RESPONSE SURFACE CONTOURS IN PERCENTAGE TOTAL HATCH

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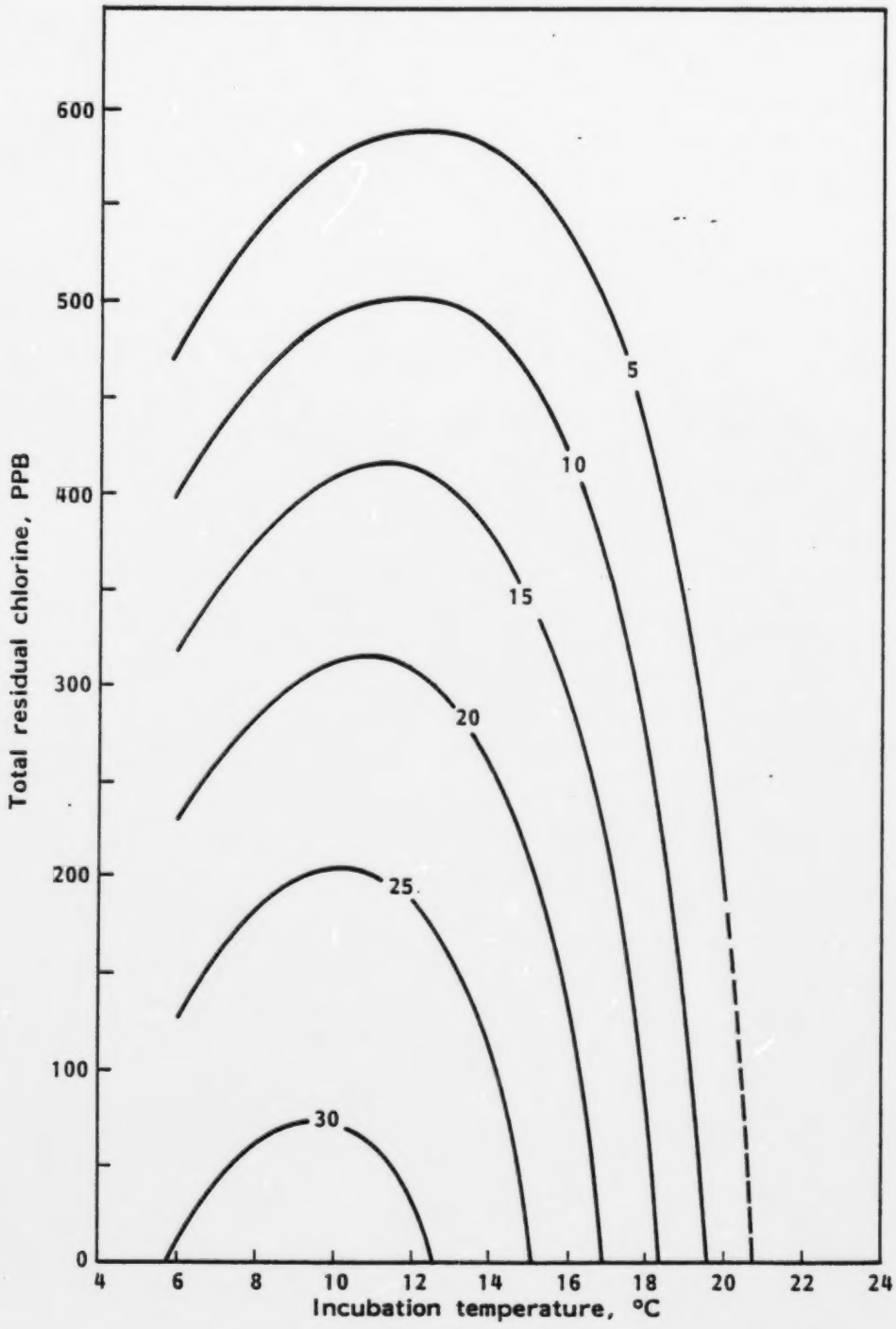

FIGURE 6

EFFECTS OF CHLORINE AND TEMPERATURE ON VIABLE HATCH OF SMELT EGGS. RESPONSE SURFACE CONTOURS IN PERCENTAGE VIABLE HATCH

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Losses which contribute to the results in Figure 6 include mortality of eggs during development (considered in the Block 1 and Block 2 models), deformed larvae and mortality of post-hatch larvae. Effects of temperature and chlorine on deformity and post-hatch mortality will now be considered in detail.

6. Larval Deformity

Smelt larvae which showed obvious deformity (primarily gross spinal curvature) were considered to be non-viable. Although most were still alive at the completion of the experiment, the deformed larvae were generally incapable of swimming effectively. All deformed larvae were enumerated and results were expressed as a percentage of the total hatch in each incubation chamber (Appendix 6A). The model fitted to the results (Appendix 6B) indicates that incubation temperature significantly affected the percentage of deformed larvae, but chlorination did not. The plot of the model (Figure 7) indicates that minimal deformity (<10% of hatched larvae) was obtained over the range of temperatures (7°C to 14°C) yielding optimal egg survival. At higher temperatures, the percentage deformity increased sharply, reaching the 40% to 60% range at 20°C.

7. Mortality of Newly-Hatched Larvae

Substantial mortality of apparently normal (undeformed) larvae was noted after hatch in some treatments. Experimental procedures were such that hatched larvae were removed from each incubation chamber and examined after each daily chlorination period. Thus, after hatch, larvae were subjected to a single two-hour chlorine dose. It was suspected that this single chlorine dose was largely responsible for the observed mortality, as the newly hatched fry were not protected by the chorion (egg membrane) which was present during embryonic development.

The numbers of apparently normal (undeformed) larvae which died after a single chlorine dose are listed in Appendix 7A. Results were computed as a percentage of the total (undeformed) hatch for each treatment to determine effects of chlorine and temperature on this mortality. Deformed larvae were excluded from this analysis, as it was suspected that the resistance of the deformed larvae to post-hatch chlorine/temperature stress might differ from that of normal larvae. A transformed response surface model (Appendix 7B) was fitted to the larval mortality data. As indicated by the Analysis of Variance, chlorine concentration strongly contributed to the observed mortality, while thermal influences were not significant. The contour plot of the larval mortality model (Figure 8) indicates that little mortality would be expected at TRC concentrations up to 200 ppb. At higher chlorine concentrations, mortality would increase sharply, reaching 50% in the 350 to 400 ppb TRC range. The model indicates total mortality of hatched fry at TRC levels of 450 to 500 ppb. The discrepancy between this model and the viable hatch model (Figure 6) which indicates from 5% to 10% viable hatch at these chlorine levels, is within the range of

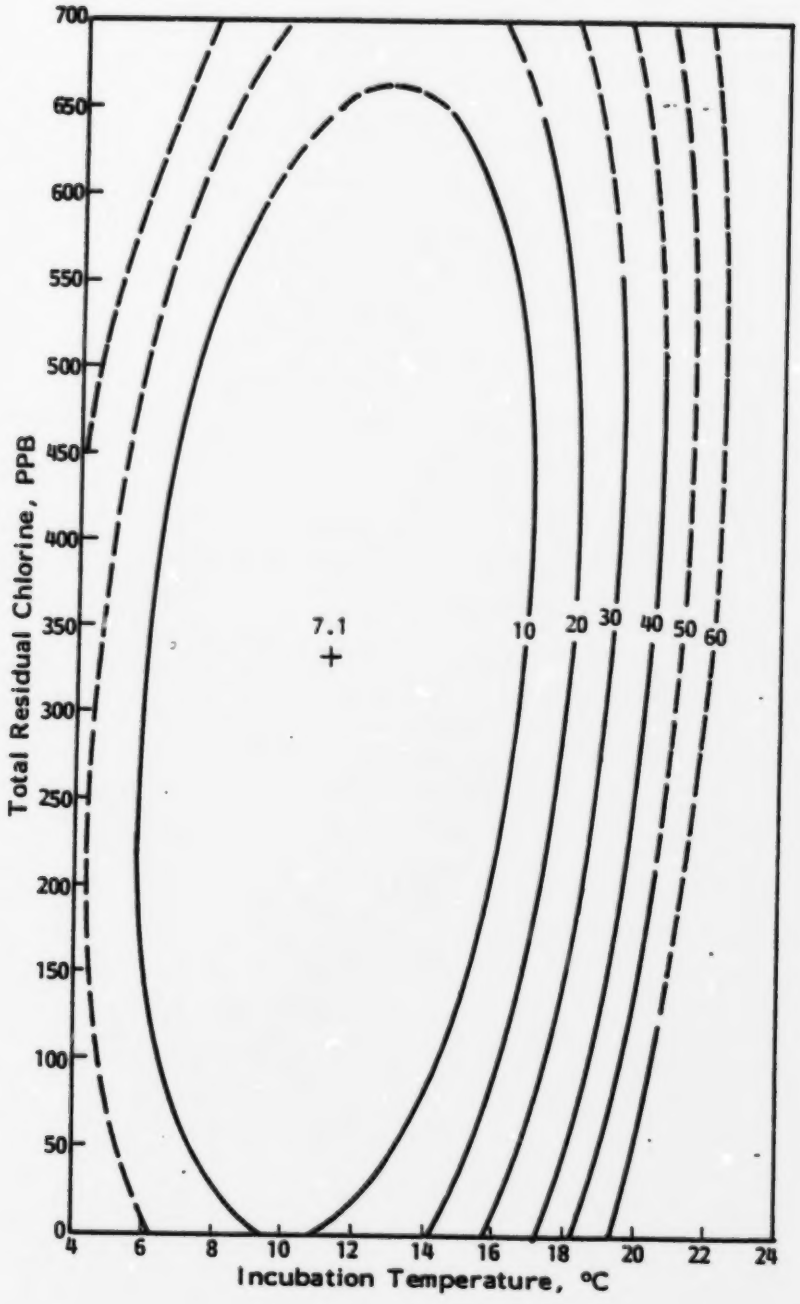

FIGURE 7

EFFECTS OF CHLORINE AND TEMPERATURE ON DEFORMITY IN DEVELOPING SMELT EMBRYOS. CONTOURS IN PERCENTAGE DEFORMITY FOR HATCHED LARVAE.

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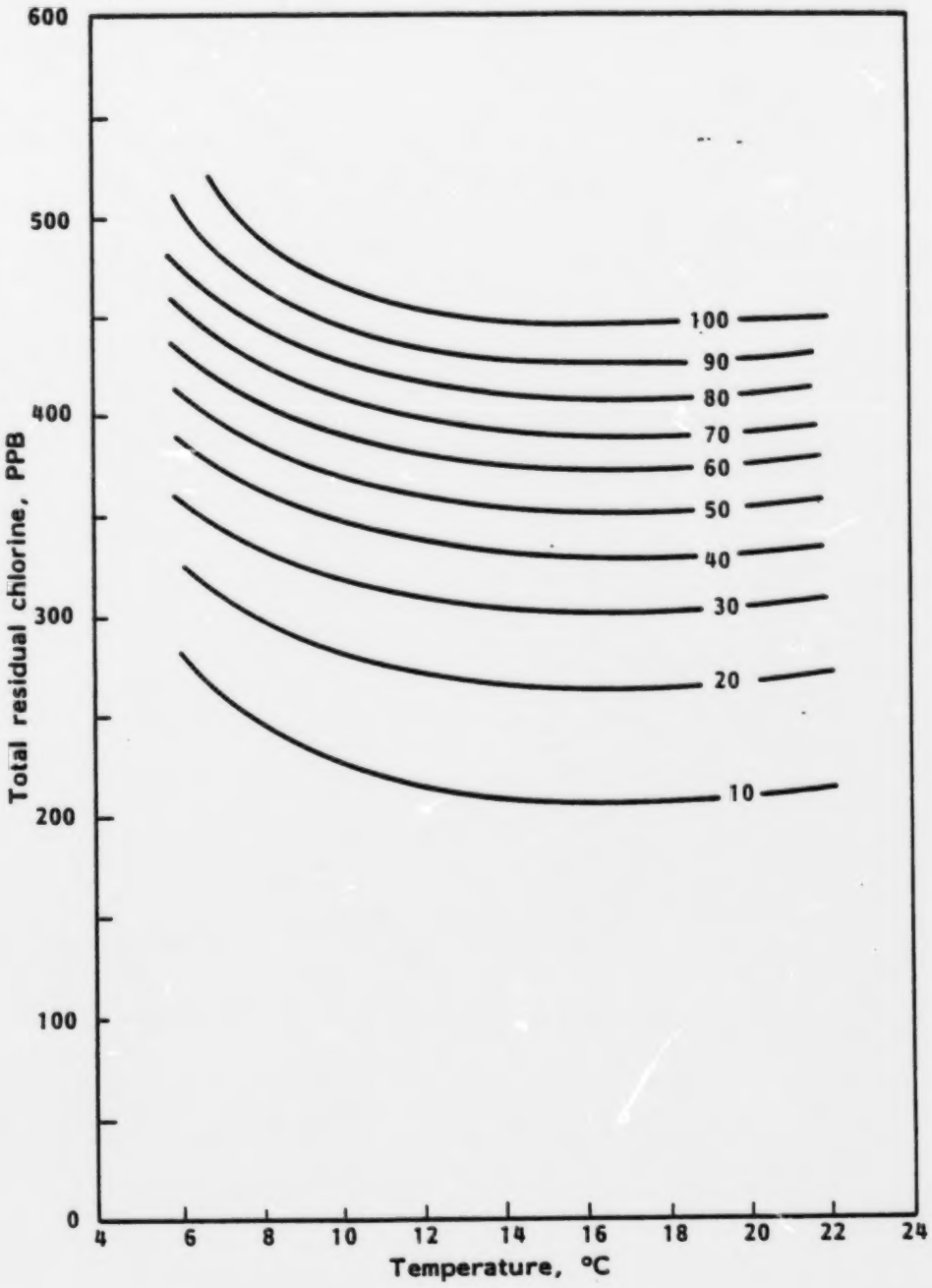

FIGURE 8

EFFECTS OF A SINGLE 2 HOUR CHLORINE EXPOSURE
ON NEWLY HATCHED SMELT LARVAE.
CONTOURS IN PERCENTAGE MORTALITY

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variability inherent in these two models (mean residuals of 8% to 9%). Figure 8 suggests a slightly enhanced chlorine toxicity with increased temperature, an effect expected due to the higher metabolic rate and hence increased chlorine uptake across the gills at warm temperatures. However, as noted in the Analysis of Variance, this effect was not statistically significant.

8. Tests With Lakeview GS Cooling Water

In addition to the previously described experiments (conducted with dechlorinated Toronto tap water) three tests (x 2 replicates) were conducted at 17°C using raw Lakeview GS cooling water as a dilution source. Nominal chlorination levels were 0, 350 and 650 ppb TRC for 2 hours/day, but as indicated in Appendix 8, measured chlorine levels were substantially below the expected dose. It is believed that this discrepancy reflects the higher chlorine demand of the rather turbid Lakeview GS condenser cooling water.

Survival data for the Lakeview condenser cooling water experiment are listed in Appendix 8. In Figure 9A, results are shown for the post-hatch mortality of larvae. It is apparent that the general pattern of results is consistent with the model obtained from the main experiment although larval mortality at the highest chlorine level (450 ppb, observed during hatching period) was somewhat less than expected. Viable hatch observed in the Lakeview cooling water was also generally comparable to that predicted from the main experiment, although mortality was greater than expected at the highest chlorine concentration (Figure 9B). Examination of the temporal mortality data for this treatment indicates that this was the result of unusually high mortality during early development (Block 1). The Block 2 mortality and losses due to deformity were near expected values for all treatments (Appendix 8).

DISCUSSION

1. Comparison With Other Studies

The effects of both constant and fluctuating temperatures on smelt egg survival were defined in a previous study in this laboratory (Ontario Hydro Research Division Report No 78-343-K). Control survival values (0 ppb chlorine) obtained in the present study for both the individual (Block 1 and Block 2) models and the summary model are very similar to results obtained at constant temperatures in the previous study. In the present study, an optimum temperature near 12°C is indicated, yielding a total hatch of 33%. The previous study indicated a 33% survival optimum at constant temperatures over 14°C to 16°C range. Estimated survival at low temperatures (7°C) and high temperatures (20°C) were nearly identical for the two studies.

Present studies have indicated that pulse chlorination for 2 hours/day at TRC levels as high as 650 ppb did not have a significant effect on mortality of developing smelt eggs. However, newly hatched larvae were substantially more sensitive, yielding

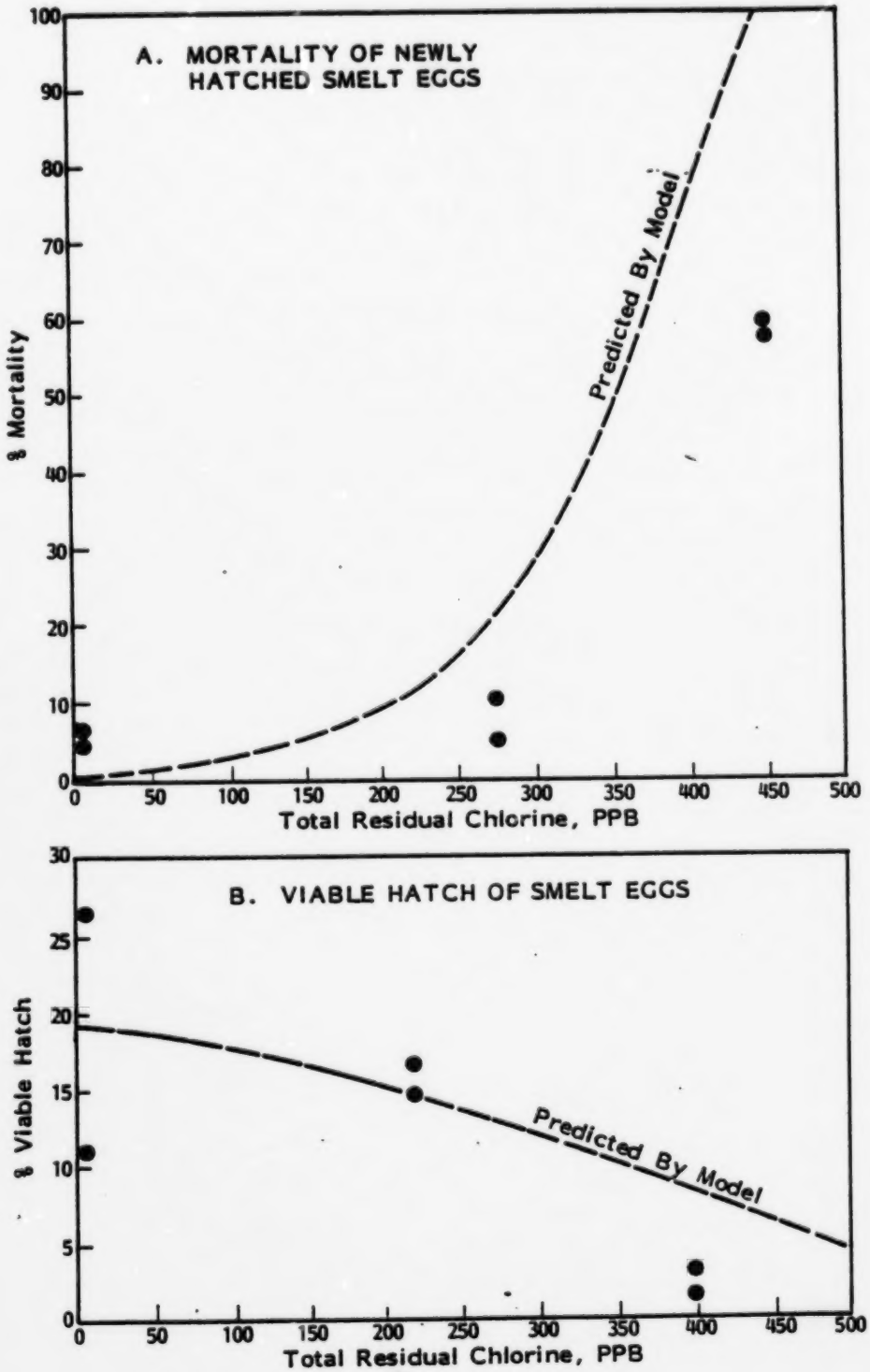

FIGURE 9

COMPARISON OF PREDICTED RESULTS AND TESTS CONDUCTED WITH CHLORINATED LAKEVIEW G.S. COOLING WATER AT 17°C

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an LC50 from 350 to 400 ppb TRC for a single two hour dose of chlorine. Few data are available in the literature on the effects of chlorine on rainbow smelt. The LC50 for a single 30-minute chlorine exposure of adult smelt was 1270 ppb, while adult smelt exposed to three 5-minute chlorine doses yielded an LC50 of 3300 ppb (Brooks and Seegert, 1977; Seegert and Brooks, 1978). These, and an avoidance value of 30 ppb (Bogardus et al, 1978) appear to be the only data reported for smelt. Seegert and Brooks (1978) suggest that chlorine tolerance of smelt is slightly greater than that of juvenile salmon (subjected to similar dosage regimes). Results of these studies cannot be compared directly with present data due to substantial differences in dosing regimes.

2. Size and Age Effects on Sensitivity to Chlorine

The present results have indicated that newly hatched smelt larvae are considerably more sensitive than incubating eggs to chlorination. Similar results have been reported for several species, but the pattern is by no means universal (see reviews by Opresko, 1980; Hall et al, 1981). Larson et al (1977) reported that coho salmon alevins were killed by TRC levels that did not affect egg survival, but early juveniles were even more sensitive than the newly hatched alevins. Alderson (1973, 1974) found that plaice eggs tolerated up to 75 ppb TRC for their 8 day incubation period, but newly hatched larvae had a 96 hour LC50 of 25 ppb. As the larvae grew, chlorine tolerance increased, with the LC50 approximating 95 ppb for 1 gram fish. Similar results were observed by Alderson for sole larvae. Burton et al (1979) reported that striped bass prolarvae in simulated entrainment studies were more sensitive than eggs to combined chlorine/temperature stress. Middaugh et al (1977) reported similar findings for striped bass eggs and larvae exposed to chlorine in seawater, and Morgan and Prince (1977) also indicated that striped bass eggs were slightly more resistant to chlorine than newly hatched larvae. In contrast, Larson et al (1977) indicated that in brook trout, newly hatched larvae were slightly more tolerant than older larvae and juveniles. Thus it appears that in most species, eggs are somewhat more resistant to chlorination than newly hatched larvae. In some species, newly hatched larvae are the most sensitive stage and resistance to chlorine increases with increasing size or age of juveniles. Salmonids, which are reasonably closely related to rainbow smelt, appear to differ somewhat. While eggs are more chlorine resistant than newly hatched larvae, the older larvae or juveniles seem to be the most chlorine-sensitive stage in these species.

3. Effects of Temperature on Sensitivity to Chlorine

Present results indicated that temperature strongly affected survival of smelt eggs, but there was no apparent interaction between chlorine and temperature. These results may be a function of the chlorine levels used, that is, different results might occur if higher chlorine concentrations were applied. Results for newly hatched smelt larvae suggest increased mortality at higher temperatures, but these thermal effects were not statistically significant. However, several authors have noted substantial increases in chlorine toxicity at warm temperatures.

Brooks and Seegert (1978a, 1978b) and Seegert et al (1979) reported declines in LC50 values with increased temperatures for spotfin, emerald and common shiners, carp, white sucker, yellow perch, bluegills and alewife.

Lethal concentrations of chlorine appear to oxidize haemoglobin, leading to death by anoxia (Grothe and Eaton, 1975). Similar results were reported by Buckley et al (1976). Cairns et al (1975a, 1975b) indicated that exposure to chlorine causes the gill epithelium to slough off, plus the production of excess mucus by the gills. Again, the end result of this process is death by asphyxia. Cherry and Cairns (1982) suggest that damage to the gills is largely caused by free chlorine, whereas chloramines cause oxidation of haemoglobin. Given the increased metabolic demands associated with high temperature, greater mortality would be expected at warm temperatures regardless of the specific mechanism of death.

4. Potential Effects of Chlorinated Thermal Discharges

While some discrepancies exist, present tests with Lakeview GS cooling water indicate that the results of the main experiment (using dechlorinated Toronto tap water) will provide reasonable predictions of effects in the Lakeview GS thermal discharge, and presumably at other Great Lakes generating station sites as well. Generally, these cooling waters will have a higher chlorine demand than the dilution water used in present experiments, more rapidly decreasing TRC levels as the cooling water discharge becomes mixed with lake water.

In addition to the variables previously discussed (species, life stage, temperature) the effects of a chlorinated discharge on fish will be dependent upon the chlorine species present in the discharge water. Brooks et al (1982) have demonstrated that for three freshwater fishes, monochloramine is the least toxic chlorine form, followed by hypochlorite, dichloramine and hypochlorous acid. In the present study, only total residual chlorine was measured. Chlorine species were not determined. However, analysis of the dechlorinated laboratory water and the batches of Lakeview GS cooling water used in this study indicated ammonia nitrogen levels approximating 30 ppb. As chlorine and NH_3 rapidly combine (in a 5:1 ratio by weight) to form monochloramine (Opresko, 1980) it would be expected that at TRC levels up to 150 ppb, most of the residual chlorine would be in this form. In discussion with CTS staff, it was indicated that residual chlorine in the Lakeview GS discharge was almost entirely monochloramine (R. Pake, personal communication). At the higher chlorine concentrations used in the present study (pH approximately 7.5 to 8.0) the test water was likely a mixture of monochloramine, hypochlorite and some hypochlorous acid.

In Figure 10, present results for smelt larvae (120 minute exposure at 15°C) are compared with LC50 levels of rainbow trout, channel catfish and emerald shiners exposed to monochloramine for various periods (Brooks et al, 1982). Data are also shown

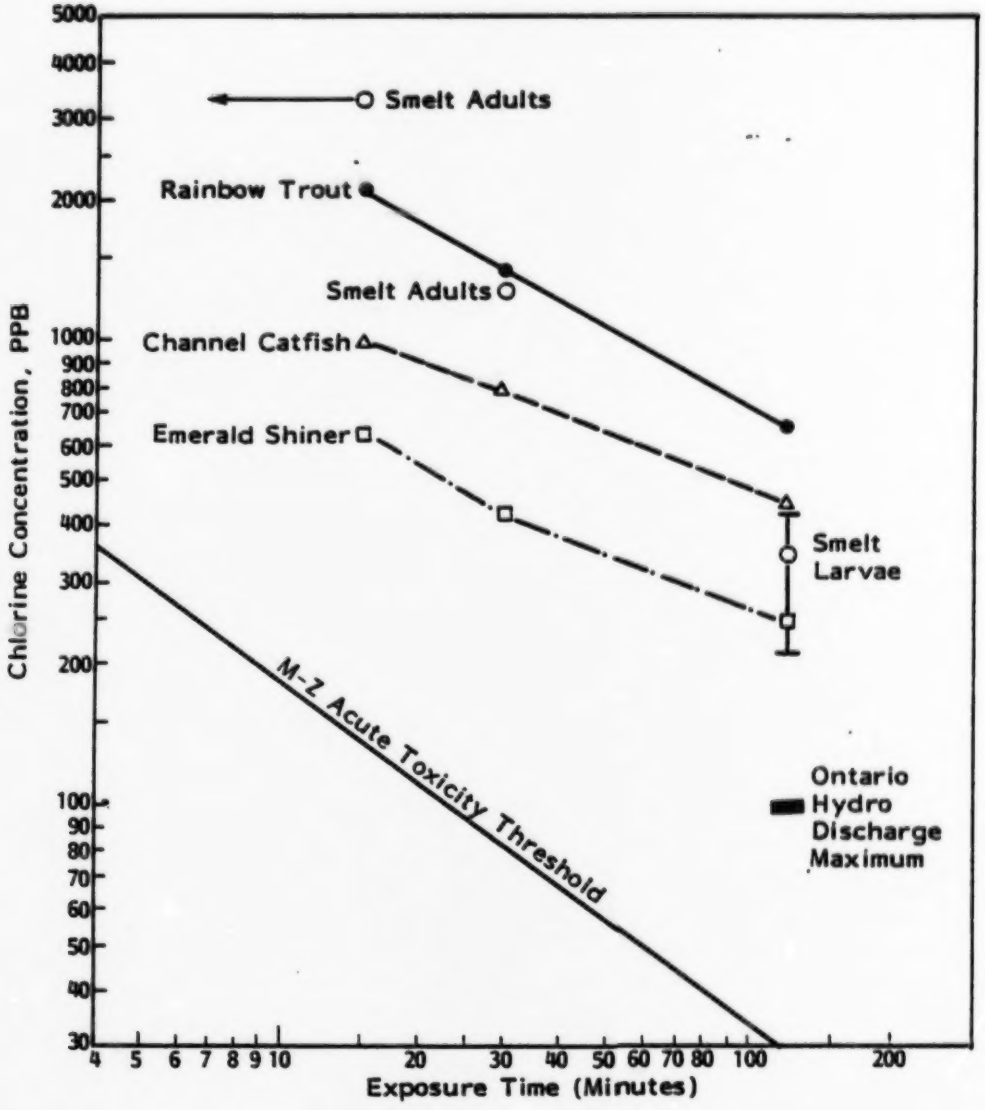

FIGURE 10

EFFECTS OF EXPOSURE TIME ON LC50 VALUES OF SEVERAL FISH SPECIES EXPOSED TO MONOCHLORAMINE

for adult smelt exposed (to primarily monochloramine) for 30 minutes (Seegert and Brooks, 1978) and 15 minutes (Brooks and Seegert, 1977). As the latter point was the result of three 5-minute exposures, it is likely equivalent to a somewhat shorter continuous exposure (note arrow in Figure 10). The results indicate that resistance of adult smelt to monochloramine is comparable to that of rainbow trout, whereas smelt larvae tested in the present study were considerably less resistant. However, test solutions in the present study likely contained some hypochlorite and hypochlorous acid at the TRC levels yielding larval mortality, thus contributing to the apparent sensitivity of smelt larvae. Under conditions which resulted in an abundance of hypochlorous acid (low ammonia, pH 6.5), Brooks et al (1982) reported 120-minute LC50 values ranging from 100 to 200 ppb for emerald shiner, channel catfish and rainbow trout.

The maximum chlorine concentration expected in Ontario Hydro cooling waters (discharged for up to 120 minutes) is substantially above the "acute toxicity threshold" (no-effect level) proposed by Mattice and Zittel (1976). However, the maximum Hydro discharge level is well below the LC50 values of any of the species considered in Figure 10. The vertical bars for the smelt larvae data indicate the LC10 to LC90 range defined in the present study and it is apparent that these values are well above the Hydro discharge maximum. The "no mortality" level for emerald shiners (the most sensitive species in Figure 10) was estimated at 170 ppb monochloramine by Brooks et al (1982). Thus, it may be concluded that if chlorine in Ontario Hydro discharges is primarily in the form of monochloramine (as is believed to be the case) then maximum discharge levels should not be acutely toxic to smelt larvae and most other fish species likely to be present in discharge plumes. Smelt eggs appear to be more resistant to chlorine than any of the fish species considered in Figure 10.

It should be noted that in the field, not all smelt larvae hatched in a thermal discharge would be exposed to maximum chlorine concentrations after hatch. Smelt larvae swim up into the water column towards the surface shortly after hatch. In a thermal discharge, these larvae would be carried away from the immediate discharge area by the thermal plume currents. Thus only those smelt larvae hatching during or slightly before the daily two hour chlorination period would be exposed (as newly hatched larvae) to the chlorine pulse.

SUMMARY

1. Rainbow smelt eggs were subjected to pulse chlorination for 2 hours/day at levels from 0 to 650 ppb TRC and temperatures from 7°C to 20°C to determine the combined effects of chlorination and thermal stress on egg survival and hatching. Experiments were run from the time of fertilization until completion of hatch (8 days at 20°C; 33 days at 7°C).

2. Results from this experiment were used to fit response surface models describing the effects of chlorine and temperature

on smelt eggs and newly hatched larvae. Due to temporal changes in mortality, separate models were fitted to results for various phases of embryonic development.

3. The models indicated that during the egg phases, temperature significantly affected survival, with optimal survival (>30%) occurring over the 8°C to 16°C range. At 20°C, survival declined to less than 20%. Pulse chlorination had no significant effect on smelt egg survival. Slightly better egg survival at high chlorination levels suggests that pulse chlorination at levels up to 650 ppb TRC for 2 hours/day may have had a beneficial biocidal effect. At these chlorination levels, the egg membrane (chorion) appeared to protect the smelt embryos from detrimental effects.

4. The viable hatch of smelt eggs (the number of live, apparently healthy larvae as opposed to total hatch) was significantly affected by both temperature and chlorine concentration. The model indicated optimal viable hatch (>30%) over the 7°C to 12°C range when TRC concentrations were less than 100 ppb. Viable hatch was sharply reduced as temperatures approached 20°C. Chlorine concentrations up to 100 ppb (maximum expected in generating station discharges) had little effect on viable hatch. Substantial decreases in viable hatch were indicated for chlorine concentrations above 200 ppb, while at 400 ppb, viable hatch was reduced to less than 50% of that expected in absence of chlorine. The production of deformed larvae and post-hatch mortality of apparently normal larvae were the two causes of difference between the total hatch and the viable hatch results.

5. Larval deformity was minimal (<10% of hatched larvae) over the range of temperatures yielding near-optimal survival (10°C to 14°C). The percentage deformity increased sharply (40% to 60% range) as temperatures approached 20°C. Chlorination did not significantly affect the production of deformed larvae.

6. Substantial numbers of apparently normal smelt larvae died shortly after hatch, following exposure to a single two-hour chlorination period. The model fitted to the larval mortality data indicated that this mortality was minimal (<10%) at TRC levels up to 200 ppb. At least 50% of normal newly hatched larvae died after exposure to 350 to 400 ppb. The model suggested that the sensitivity to chlorine was greater at warm temperatures. Such results would be expected on the basis of metabolic rate increases and the mechanisms of chlorine toxicity (which involve death by asphyxia) but this trend was not statistically significant.

7. A small series of tests was run at 17°C (0 to 650 ppb TRC) using Lakeview GS discharge water as a dilution source.

Comparison with models from the main experiment (using dechlorinated Toronto tap water) indicated that while some discrepancies existed, the Lakeview GS results generally fell within the range of values predicted by the models. Thus, results of the present study should be applicable to generating stations using Great Lakes waters as a cooling source.

8. Results from this study indicate that the maximum chlorine concentration expected in Ontario Hydro thermal discharges (100 ppb for 2 hours) is well below toxic levels for newly hatched smelt larvae. Recent data in the literature indicate that other chlorine-sensitive species (rainbow trout, channel catfish, emerald shiner) also would not suffer mortality from these levels if the chlorine is primarily in the form of monochloramine.

The laboratory work in this study, including construction of apparatus, conducting the experiments, chlorine analysis and computer analysis of results was done by Mr. P.U. Niklas and Ms. G.A. Watson.

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REFERENCES

ALDERSON, R. 1973. Effects of low concentrations of free chlorine on eggs and larvae of plaice, Pleuronectes platessa L. In: M. Ruvio (ed). Marine pollution and sea life. Fishing News (Books) Ltd., London. pp 311-315.

ALDERSON, R. 1974. Seawater chlorination and the survival and growth of the early developmental stages of plaice, Pleuronectes platessa L., and Dover sole, Solea solea (L.). Aquaculture 4:41-53.

BOGARDUS, R.B., T.C. TEPPEN, D.B. BOIES, and F.J. HORVATH. 1978. Avoidance of monochloramine: test tank results for rainbow smelt, coho salmon, alewife, yellow perch, and spotted shiner. In: R.L. Jolley, H. Gorchev, and D.H. Hamilton (eds.) Water chlorination: environmental impact and health effects, Vol. 2. Ann Arbor Science Publishers, Ann Arbor, Michigan. pp 149-161.

BROOKS, A.S. and G.L. SEEGERT. 1977. The effects of intermittent chlorination on the biota of Lake Michigan. Special Report No. 31. University of Wisconsin Center for Great Lakes Studies, Milwaukee. 167 pp.

BROOKS, A.S. and G.L. SEEGERT. 1978a. A preliminary look at the effects of intermittent chlorination on selected warmwater fishes. In: R.L. Jolley, H. Gorchev and D.H. Hamilton (eds.) Water chlorination: environmental impact and health effects, Vol. 2. Ann Arbor Science Publishers, Ann Arbor, Michigan. pp 95-109.

BROOKS, A.S. and G.L. SEEGERT. 1978b. The effects of intermittent chlorination on ten species of warmwater fish. Special Report No. 35. University of Wisconsin Center for Great Lakes Studies, Milwaukee.

BROOKS, A.S., J.M. BARTOS and P.T. DANOS. 1982. The effects of chlorine on freshwater fish under various time and chemical conditions. Electric Power Research Institute, Palo Alto, California. Report No EA-2481.

BUCKLEY, J.A., C.M. WHITMORE and R.I. MATSUDA. 1976. Changes in blood chemistry and blood cell morphology in coho salmon (Oncorhynchus kisutch) following exposure to sublethal levels of total residual chlorine in municipal wastewater. J. Fish. Res. Board Can. 33:776-782.

BURTON, D.T., L.W. HALL, JR., S.L. MARGREY, and R.D. SMALL. 1979. Interactions of chlorine, temperature change (ΔT), and exposure time on survival of striped bass (Morone saxatilis) eggs and prolarvae. J. Fish. Res. Board Can. 36: 1108-1113.

BUTLER, F., R. KOZOPAS, G.T. HAYMES and J.S. GRIFFITHS. 1981. Water chlorination programs at Ontario Hydro fossil-fired generating stations. Proceedings of 4th Conference on Water Chlorination. Environmental and Health Effects. Pacific Grove, California. October, 1981.

CAIRNS, J. JR., A.G. HEATH and B.C. PARKER. 1975a. Temperature influence on chemical toxicity to aquatic organisms. J. Water Poll. Control Fed. 47:267.

CAIRNS, J. JR., A.G. HEATH and B.C. PARKER. 1975b. The effects of temperature upon the toxicity of chemicals to aquatic organisms. Hydrobiologia 47: 135-171.

CHERRY, D.S. and J. CAIRNS, JR. 1982. Biological monitoring. Part V - preference and avoidance studies. Water Res. 16:263-301.

GROTHE, D.R. and J.W. EATON. 1975. Chlorine-induced mortality in fish. Trans. Am. Fish. Soc. 104:800-802.

HALL, L.W. JR., G.R. HELZ and D.T. BURTON. 1981. Power plant chlorination. A biological and chemical assessment. Electric Power Research Institute, Palo Alto, California. Report No. EA-1750. 237 pp.

HERRMANN, O. 1981. Personal communication.

LARSON, G.L., F.E. HUTCHINS and L.P. LAMPERTI. 1977. Laboratory determinations of acute and sublethal toxicities of inorganic chloramines to early life stages of coho salmon. Trans. Am. Fish. Soc. 106:268-277.

LINDSEY, J.K., D.F. ALDERDICE and L.V. PIENAAR. 1970. Analysis of nonlinear models - the nonlinear response surface. J. Fish. Res. Board Can. 27:765-791.

LINDSEY, J.K. and A.M. SANDNES. MS 1972. Program for the analysis of nonlinear response surfaces (version III). Fish Res. Board Can. Tech. Rep. No. 311. 145 pp.

MATTICE, J.S. and H.E. ZITTEL. 1976. Site specific evaluation of power plant chlorination. J. Wat. Pollut. Control Fed. 48:2284-2308.

MIDDAUGH, D.P., J.A. COUCH and A.M. CRANE. 1977. Responses of early life history stages of the striped bass Morone saxatilis to chlorination. Chesapeake Sci. 18:141-153.

MORGAN, R.P. II and R.D. PRINCE. 1977. Chlorine toxicity to eggs and larvae of five Chesapeake Bay fishes. Trans. Am. Fish. Soc. 106:380-385.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 75-21-H. Free and combined chlorine in condenser cooling water. L.E. Johnston, January 15, 1975.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 75-484-K. The use of nonlinear response surface models in the analysis of experimental data. J.S. Griffiths, November 25, 1975.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 78-28-K. Field fish spawning study, Lennox 1976. W.E. Dunford, January 23, 1978.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 78-343-K. Potential effects of unstable thermal discharge plumes on Great Lakes rainbow smelt eggs. J.S. Griffiths, July 19, 1978.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 80-123-K. Fish spawning in chlorine plumes at three thermal generating stations. G.T. Haymes, May 23, 1980.

ONTARIO HYDRO RESEARCH DIVISION REPORT NO. 82-59-K. Nanticoke TGS tempering exemption study, biological investigations, May 1980, to March 1981. H. Balesic, August 4, 1982.

OPRESKO, D.M. 1980. Review of open literature on effects of chlorine on aquatic organisms. Electric Power Research Institute, Palo Alto, California. Report No. EA-1491.

PAKE, R. 1981. Personal communication.

SCOTT, W.B. and E.J. CROSSMAN. 1973. Freshwater fishes of Canada. Bulletin 184, Fish. Res. Board Can. 966 p.

SEEGERT, G.L. and A.S. BROOKS. 1978. The effects of intermittent chlorination on coho salmon, alewife, spottail shiner and rainbow smelt. Trans. Am. Fish. Soc. 107: 346-353.

SEEGERT, G.L., A.S. BROOKS, J.R. VANDE CASTLE and K. GRADALL. 1979. The effects of monochloramine on selected riverine fishes. Trans. Am. Fish. Soc. 108:88-96.

APPENDIX 1

DEVELOPMENTAL STAGES IN SMELT

Stage		Description
1	Small Blastodisc	Multicellular polar cap
2	Large Blastodisc	Flattened thin undifferentiated cap
3	Germ Ring and Embryonic Shield	Peripheral band of cells beginning to overgrow yolk, inwardly directed mass of cells
4	One-Quarter Epiboly	Germ ring has overgrown one-fourth of yolk, embryonic shield elongating
5	One-Half Epiboly	Germ ring has overgrown one-half of yolk, cephalic differentiation beginning
6	Three-Quarter Epiboly	Germ ring has overgrown three-quarters of yolk, somites differentiating
7	Blastopore Closing	Small drop-shaped space within germ ring
8	Blastopore Closed	Segmented trunk ending in a node
9	Trunk Free of Sac	Trunk has grown beyond yolk sac and is beginning to coil
*10	Trunk One-Quarter Final Length	Tip of tail bud close to head but not overlapping
*11	First Eye Pigment	Optic cup two layered, faint pigment visible around lens
*12	Trunk half Final Length	Tip of tail bud overlapping head by half its length
*13	Trunk Final Length	Free portion of trunk curved over a full 360 degree circle
14	Medium Eye Pigment	Eye pigment strongly visible, but not completely opaque
15	Complete Eye Pigment	Eye pigment completely opaque
16	Notochord Upturned	Posterior tip of notochord and vertebral column flexing caudodorsad
17	Caudal Fin Forming	Fin-fold taking on the shape of caudal fin
18	Body Pigment Complete	Melanophores along ventral surface
19	Pre-Hatch	Pronounced movement of embryo
20	Hatch	Larvae hatch and swim to surface

Above stages based on Garside's (1959) classification for lake trout embryos.

*Stages modified from Garside's classification - vitelline venation and fin-fold (used by Garside) were not clearly visible in smelt embryos.

APPENDIX 2A

TEMPERATURE/CHLORINE BIOASSAY OF RAINBOW SMELT EGGS
DATA FOR BLOCK 1 (FERTILIZATION TO STAGE 8)

Experimental Conditions				Results		
Design		Observed ± C.I.			Embryo Survival	
Temp °C	TRC ppb	Temp °C	TRC ppb	Time (Days)	Initial No	Final No	% Survival
7	0	6.69 ± 0.15	59.75 ± 4.51	8	440	156	35.45
		6.55 ± 0.10	59.75 ± 4.51		494	91	18.42
	50	6.52 ± 0.10	84.39 ± 9.95	8	574	151	26.31
		6.47 ± 0.10	84.39 ± 9.95		470	158	32.62
	230	6.48 ± 0.11	239.60 ± 21.96	7	468	200	42.74
		6.42 ± 0.10	239.60 ± 21.96		506	245	48.42
7	490	6.60 ± 0.10	499.40 ± 31.94	7	554	264	47.65
		6.56 ± 0.09	499.40 ± 31.94		484	226	46.69
	0	11.78 ± 0.32	47.71 ± 4.22	3	376	201	53.46
		11.72 ± 0.12	47.71 ± 4.22		352	189	53.69
	130	11.55 ± 0.13	157.80 ± 0.00	3	344	220	63.95
		11.53 ± 0.09	157.80 ± 0.00		598	227	37.96
12	350	11.55 ± 0.09	379.95 ± 98.20	3	691	286	41.39
		11.68 ± 0.15	379.95 ± 98.20		343	124	36.15
	650	11.58 ± 0.12	615.70 ± 0.00	3	601	333	55.41
		11.60 ± 0.12	615.70 ± 0.00		687	209	30.42
	0	16.28 ± 0.15	52.93 ± 25.92	2	255	130	50.98
		16.35 ± 0.09	52.93 ± 25.92		383	50	13.05
17	130	16.35 ± 0.09	131.04 ± 48.47	2	651	219	33.64
		16.35 ± 0.09	131.04 ± 48.47		632	129	20.41
	350	16.30 ± 0.13	344.80 ± 80.74	2	893	207	23.18
		16.33 ± 0.08	344.80 ± 80.74		809	267	33.00
	650	16.38 ± 0.08	613.70 ± 93.09	2	630	335	53.17
		16.38 ± 0.08	651.40 ± 45.25		511	334	65.36
0	0	19.53 ± 0.54	43.66 ± 55.65	2	136	79	58.09
		19.53 ± 0.54	43.66 ± 55.65		745	41	5.50
0	50	19.55 ± 0.46	49.67 ± 139.70	2	145	43	29.66
		19.50 ± 0.56	63.31 ± 33.60		218	128	58.72
	230	19.43 ± 0.60	203.60 ± 193.96	2	293	80	27.30
		19.45 ± 0.67	203.60 ± 193.96		377	28	7.43
0	490	19.48 ± 0.61	437.60 ± 64.61	2	372	129	34.68
		19.50 ± 0.57	437.60 ± 64.61		253	86	33.99

APPENDIX 2B

ANALYSIS OF SMELT EGG BLOCK 1 SURVIVAL DATA

Analysis of Variance Table - Untransformed Data

Source	SS	DF	MSS	F
Regression	1561.8367	5	312.37	1.33
Linear	614.7114	2	307.36	1.31
x_1 Linear	254.4770	1	254.48	1.08
x_2 Linear	334.7565	1	334.76	1.43
Quadratic	808.7531	2	404.38	1.72
x_1 Quad	253.6837	1	253.68	1.08
x_2 Quad	454.4713	1	454.47	1.94
$x_1 * x_2$	29.4490	1	29.45	0.13
Residual	6098.3325	26	234.55	
TOTAL	7660.1692	31		

Regression:

$$Y = 21.5331 + 3.88069x_1 - .044157x_2 - .161928x_1^2 + .000119721x_2^2 - .00109805x_1x_2$$

Y = % Survival

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Residual for Model (32 data points) = 10.1%

APPENDIX 3A

TEMPERATURE/CHLORINE BIOASSAY OF RAINBOW SMELT EGGS
DATA FOR BLOCK 2 (STAGE 8 TO COMPLETION OF HATCH)

Experimental Conditions					Results		
Design		Observed ± C.I.			Embryo Survival		
Temp °C	TRC ppb	Temp °C	TRC ppb	Time (Days)	Initial No	Final No	% Survival
7	0	7.05 ± 0.07	57.90 ± 3.03	25	156	103	66.03
		6.96 ± 0.08	57.90 ± 3.03	24	91	86	94.51
7	50	6.89 ± 0.07	81.82 ± 2.61	25	150	115	76.67
		6.88 ± 0.07	81.82 ± 2.61	25	158	139	87.97
	230	6.79 ± 0.07	238.20 ±12.00	26	200	172	86.00
		6.77 ± 0.08	238.20 ±12.00	26	244	226	92.62
7	490	7.01 ± 0.08	510.90 ±24.20	26	263	185	70.34
		6.94 ± 0.07	510.90 ±24.20	26	226	208	92.03
12	0	11.80	47.27	Omitted due to escape of larvae			
		11.80 ± 0.12	47.27 ± 4.77	13	189	154	81.48
	130	11.84 ± 0.09	161.90 ± 5.81	13	220	202	91.82
		11.85 ± 0.09	161.90 ± 5.81	13	226	183	80.97
12	350	11.79 ± 0.07	391.40 ±11.39	15	284	230	80.99
		11.79 ± 0.07	391.40 ±11.39	13	123	113	91.87
12	650	11.88 ± 0.07	622.50 ±20.83	15	333	305	91.59
		11.92 ± 0.08	622.50 ±20.83	15	207	134	64.73
17	0	16.83 ± 0.21	52.17 ± 3.07	9	130	73	56.15
		16.78 ± 0.20	52.17 ± 3.07	8	50	43	86.00
17	130	16.85 ± 0.20	136.30 ±21.94	9	218	171	78.44
		16.80 ± 0.21	136.30 ±21.94	8	129	88	68.22
17	350	16.76 ± 0.18	367.00 ±18.29	9	207	156	75.36
		16.76 ± 0.18	367.00 ±18.29	9	266	249	93.61
17	650	16.82 ± 0.16	636.70 ±20.77	9	335	253	75.52
		16.86 ± 0.15	639.10 ±22.90	9	334	203	60.78
20	0	20.36 ± 0.90	40.99 ± 2.71	5	76	43	56.58
		20.37 ± 0.90	40.99 ± 2.71	5	41	21	51.22
20	50	20.59 ± 1.04	56.63 ±16.79	5	43	34	79.07
		20.34 ± 0.91	53.63 ±14.84	4	124	77	62.10
	230	20.38 ± 0.83	201.60 ±10.61	6	80	42	52.50
		20.30 ± 0.90	201.60 ±10.61	5	28	7	25.00
20	490	20.30 ± 0.88	490.20 ±41.60	5	129	52	40.31
		20.39 ± 0.81	490.20 ±41.60	6	86	47	54.65

APPENDIX 3B

ANALYSIS OF SMELT EGG BLOCK 2 SURVIVAL DATA

Analysis of Variance Table - Untransformed Data

Source	SS	DF	MSS	F
Regression	4689.2037	5	937.84	5.78*
Linear	3242.5116	2	1621.26	9.99*
x_1 Linear	3205.6308	1	3205.63	19.76*
x_2 Linear	64.9043	1	64.90	0.40
Quadratic	1400.8759	2	700.44	4.32*
x_1 Quad	1346.8117	1	1346.81	8.30*
x_2 Quad	87.2229	1	87.22	0.54
$x_1 * x_2$	154.8415	1	154.84	0.95
Residual	4056.5099	25	162.26	
TOTAL	8745.7136	30		

Regression:

$$Y = 35.4894 + 8.19587x_1 + .0593753x_2 - .354439x_1^2 - .0000533576x_2^2 - .00230679x_1x_2$$

Y = % Survival

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Residual for Model (31 data points) = 8.97%

*Indicates significance at 0.05 probability level.

APPENDIX 4A

TEMPERATURE/CHLORINE BIOASSAY OF SMELT EGGS
DATA FOR TOTAL HATCH (FERTILIZATION TO COMPLETE HATCH)

Experimental Conditions					Results		
Design		Observed ± C.I.			Embryo Survival		
Temp °C	TRC ppb	Temp °C	TRC ppb	Time (Days)	Initial No	Final No	% Hatch
7	0	6.97 ± 0.08	58.35 ± 2.45	33	440	103	23.41
		6.86 ± 0.09	58.35 ± 2.45	32	494	86	17.41
7	50	6.80 ± 0.07	82.44 ± 2.80	33	574	115	20.03
		6.78 ± 0.07	82.44 ± 2.80	33	470	139	29.57
7	230	6.73 ± 0.07	238.50 ± 10.02	33	468	172	36.75
		6.69 ± 0.07	238.50 ± 10.02	33	506	226	44.66
7	490	6.92 ± 0.07	508.50 ± 19.63	33	554	185	33.39
		6.86 ± 0.07	508.50 ± 19.63	33	484	208	42.98
12	0	11.78 ± 0.10	47.35 ± 3.78	16	352	154	43.75
12	130	11.78 ± 0.08	161.10 ± 4.66	16	344	202	58.72
		11.78 ± 0.08	161.10 ± 4.66	16	598	183	30.60
12	350	11.75 ± 0.06	389.50 ± 11.68	18	691	230	33.29
		11.77 ± 0.06	389.50 ± 11.68	16	343	113	32.94
12	650	11.83 ± 0.07	621.40 ± 17.02	18	601	305	50.75
		11.87 ± 0.08	621.40 ± 17.02	18	687	134	19.51
17	0	16.72 ± 0.20	52.32 ± 2.49	11	255	73	28.63
		16.69 ± 0.18	52.32 ± 2.49	10	383	43	11.23
17	130	16.75 ± 0.19	135.30 ± 17.24	11	651	171	26.27
		16.71 ± 0.19	135.30 ± 17.24	10	632	88	13.92
17	350	16.67 ± 0.17	362.90 ± 15.63	11	893	156	17.47
		16.68 ± 0.16	362.90 ± 15.63	11	809	249	30.78
17	650	16.74 ± 0.15	632.90 ± 26.55	11	630	253	40.16
		16.75 ± 0.14	641.30 ± 21.12	11	511	203	39.73
20	0	20.12 ± 0.65	41.75 ± 3.10	7	136	43	31.62
		20.13 ± 0.65	41.75 ± 3.10	7	745	21	2.82
20	50	20.27 ± 0.74	56.05 ± 10.74	7	145	34	23.45
		20.10 ± 0.66	54.64 ± 12.19	6	218	77	35.32
20	230	20.13 ± 0.64	202.10 ± 9.90	8	293	42	14.33
		20.06 ± 0.66	202.10 ± 9.90	7	377	7	1.86
20	490	20.06 ± 0.64	477.10 ± 34.70	7	372	52	13.98
		20.15 ± 0.61	477.10 ± 34.70	8	253	47	18.58

APPENDIX 4B

ANALYSIS OF SMELT EGG BLOCK 3 (TOTAL HATCH) SURVIVAL DATA

Analysis of Variance Table - Untransformed Data

Source	SS	DF	MSS	F
Regression	1878.9972	5	375.80	2.66*
Linear	1248.0814	2	624.04	4.42*
x_1 Linear	1047.4065	1	1047.41	7.41*
x_2 Linear	167.0957	1	167.10	1.18
Quadratic	455.0374	2	227.52	1.61
x_1 Quad	391.8083	1	391.81	2.77
x_2 Quad	44.0371	1	44.04	0.31
$x_1 * x_2$	100.2947	1	100.29	0.71
Residual	3532.1699	25	141.29	
TOTAL	5411.1670	30		

Regression:

$$Y = 6.19546 + 4.60285x_1 + .0140105x_2 - .197017x_1^2 + .0000378162x_2^2 - .00190152x_1x_2$$

Y = % Survival

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Residual for Model (31 data points) = 8.48%

*Indicates significance at 0.05 probability level.

APPENDIX 5A

TEMPERATURE/CHLORINE BIOASSAY OF SMELT EGGS
DATA FOR VIABLE HATCH (FERTILIZATION TO COMPLETE HATCH)

Experimental Conditions					Results		
Design		Observed ± C.I.			Embryo Survival		
Temp °C	TRC ppb	Temp °C	TRC ppb	Time (Days)	Initial No	Final No	% Viable Hatch
7	0	6.97 ± 0.08	58.35 ± 2.45	33	440	96	21.82
		6.86 ± 0.09	58.35 ± 2.45	32	494	73	14.78
7	50	6.80 ± 0.07	82.44 ± 2.80	33	574	97	16.90
		6.78 ± 0.07	82.44 ± 2.80	33	470	119	25.32
7	230	6.73 ± 0.07	238.50 ± 10.02	33	468	156	33.33
		6.69 ± 0.07	238.50 ± 10.02	33	506	201	39.72
7	490	6.92 ± 0.07	508.50 ± 19.63	33	554	12	2.17
		6.86 ± 0.07	508.50 ± 19.63	33	484	30	6.20
12	0	11.78 ± 0.10	47.35 ± 3.78	16	352	145	41.19
12	130	11.78 ± 0.08	161.10 ± 4.66	16	344	196	56.98
		11.78 ± 0.08	161.10 ± 4.66	16	598	181	30.27
12	350	11.75 ± 0.06	389.50 ± 11.68	18	691	6	0.87
		11.77 ± 0.06	389.50 ± 11.68	16	343	3	0.87
12	650	11.83 ± 0.07	621.40 ± 17.02	18	601	7	1.16
		11.87 ± 0.08	621.40 ± 17.02	18	687	6	0.87
17	0	16.72 ± 0.20	52.32 ± 2.49	11	255	59	23.14
		16.69 ± 0.18	52.32 ± 2.49	10	383	33	8.62
17	130	16.75 ± 0.19	135.30 ± 17.24	11	651	146	22.43
		16.71 ± 0.19	135.30 ± 17.24	10	632	75	11.87
17	350	16.67 ± 0.17	362.90 ± 15.63	11	893	55	6.16
		16.68 ± 0.16	362.90 ± 15.63	11	809	16	1.98
17	650	16.74 ± 0.15	632.90 ± 26.55	11	630	30	4.76
		16.75 ± 0.14	641.30 ± 21.12	11	511	8	1.57
20	0	20.12 ± 0.65	41.75 ± 3.10	7	136	22	16.18
		20.13 ± 0.65	41.75 ± 3.10	7	745	4	0.54
20	50	20.27 ± 0.74	56.05 ± 10.74	7	145	4	2.76
		20.10 ± 0.66	54.64 ± 12.19	6	218	26	11.93
20	230	20.13 ± 0.64	202.10 ± 9.90	8	293	5	1.71
		20.06 ± 0.66	202.10 ± 9.90	7	377	5	1.33
20	490	20.06 ± 0.64	477.10 ± 34.70	7	372	3	0.81
		20.15 ± 0.61	477.10 ± 34.70	8	253	0	0.00

APPENDIX 5B

ANALYSIS OF RAINBOW SMELT CHLORINE/TEMPERATURE BIOASSAY DATA-PERCENTAGE VIABLE HATCH

Analysis of Variance Table - Untransformed Data

Source	SS	DF	MSS	F
Regression	3503.9060	5	700.78	5.70*
Linear	3066.9652	2	1533.48	12.47*
x_1 Linear	1088.3410	1	1088.34	8.85*
x_2 Linear	2088.8597	1	2088.86	16.98*
Quadratic	461.3769	2	230.69	1.88
x_1 Quad	442.3926	1	442.39	3.60
x_2 Quad	32.7922	1	32.79	0.27
$x_1 * x_2$	135.5278	1	135.53	1.10
Residual	3074.6592	25	122.99	
TOTAL	6578.5651	30		

Regression:

$$Y = 14.867 + 3.83627x_1 - .0512633x_2 - .20933x_1^2 - .0000326325x_2^2 + .00221044x_1x_2$$

Y = % Viable Hatch

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Resdiaul for Model (31 data points) = 7.7%

*Indicates significance at 0.05 probability level.

APPENDIX 6A

TEMPERATURE/CHLORINE BIOASSAY OF RAINBOW SMELT EGGS
DATA FOR PERCENTAGE DEFORMITY OF HATCHED LARVAE

Experimental Conditions				Results		
Design		Observed ± C.I.				
Temp °C	TRC ppb	Temp °C	TRC ppb	Number Hatched	Number Deformed	% Deformity
7	0	6.97 ± 0.08	58.35 ± 2.45	103	6	5.83
		6.86 ± 0.09	58.35 ± 2.45	86	12	13.95
7	50	6.80 ± 0.07	82.44 ± 2.80	115	14	12.17
		6.78 ± 0.07	82.44 ± 2.80	139	17	12.23
7	230	6.73 ± 0.07	238.50 ± 10.02	172	15	8.72
		6.69 ± 0.07	238.50 ± 10.02	226	17	7.52
7	490	6.92 ± 0.07	508.50 ± 19.63	185	9	4.86
		6.86 ± 0.07	508.50 ± 19.63	208	12	5.77
12	0	11.78 ± 0.10	47.35 ± 3.78	154	4	2.60
12	130	11.78 ± 0.08	161.10 ± 4.66	202	5	2.48
		11.78 ± 0.08	161.10 ± 4.66	183	2	1.09
12	350	11.75 ± 0.06	389.50 ± 11.68	230	14	6.09
		11.77 ± 0.06	389.50 ± 11.68	113	3	2.65
12	650	11.83 ± 0.07	621.40 ± 17.02	305	17	5.57
		11.87 ± 0.08	621.40 ± 17.02	134	3	2.24
17	0	16.72 ± 0.20	52.32 ± 2.49	73	12	16.43
		16.69 ± 0.18	52.32 ± 2.49	43	8	18.60
17	130	16.75 ± 0.19	135.30 ± 17.24	171	23	13.45
		16.71 ± 0.19	135.30 ± 17.24	88	8	9.09
17	350	16.67 ± 0.17	362.90 ± 15.63	156	23	14.74
		16.68 ± 0.16	362.90 ± 15.63	249	19	7.63
17	650	16.74 ± 0.15	632.90 ± 26.55	253	27	10.67
		16.75 ± 0.14	641.30 ± 21.12	203	87	42.86
20	0	20.12 ± 0.65	41.75 ± 3.10	43	21	48.84
		20.13 ± 0.65	41.75 ± 3.10	21	16	76.19
20	50	20.27 ± 0.74	56.05 ± 10.74	34	30	88.24
		20.10 ± 0.66	54.64 ± 12.19	77	51	66.23
20	230	20.13 ± 0.64	202.10 ± 9.90	42	35	83.33
		20.06 ± 0.66	202.10 ± 9.90	7	1	14.29
20	490	20.06 ± 0.64	477.10 ± 34.70	52	8	15.38
		20.15 ± 0.61	477.10 ± 34.70	47	14	29.79

APPENDIX 6B

ANALYSIS OF SMELT CHLORINE/TEMPERATURE BIOASSAY DATA-PERCENTAGE DEFORMITY IN HATCHED LARVAE

Analysis of Variance Table - Untransformed Data

Source	SS	DF	MSS	F
Regression	13470.6506	5	2694.13	12.31*
Linear	6084.8804	2	3042.44	13.91*
x_1 Linear	5677.0131	1	5677.01	25.95*
x_2 Linear	299.8437	1	299.84	1.37
Quadratic	4091.5127	2	2045.76	9.35*
x_1 Quad	3462.4356	1	3462.44	15.83*
x_2 Quad	834.3197	1	834.32	3.81
$x_1 * x_2$	609.3042	1	609.30	2.79
Residual	5469.2454	25	218.77	
TOTAL	18939.8960	30		

Regression:

$$Y = 68.6394 - 11.7369x_1 - .0555574x_2 + .585625x_1^2 + .000164601x_2^2 - .00468684x_1x_2$$

Y = % Deformed Larvae

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Residual for Model (31 data points) = 9.87%

*Indicates significance at 0.05 probability level.

APPENDIX 7A

TEMPERATURE/CHLORINE BIOASSAY OF RAINBOW SMELT EGGS
DATA FOR MORTALITY OF NEWLY HATCHED (NORMAL) LARVAE

Experimental Conditions				Results			
Design		Observed ± C.I.					
Temp °C	TRC ppb	Temp °C	TRC ppb	Hatching Period (Days)	Normal Larvae Hatched	Number of Mortalities	% Mortality
7	0	7.04 ± 0.15	59.74 ± 3.38	8	97	1	1.03
		6.99 ± 0.25	60.78 ± 5.95	5	74	1	1.35
7	50	6.83 ± 0.42	82.43 ± 18.24	3	99	2	2.02
		6.89 ± 0.20	81.76 ± 5.99	6	122	3	2.46
7	230	6.83 ± 0.46	212.03 ± 81.27	3	157	1	0.64
		6.79 ± 0.15	239.17 ± 46.11	7	209	8	3.83
7	490	7.01 ± 0.12	492.65 ± 58.57	8	176	164	93.18
		6.96 ± 0.17	510.99 ± 76.00	6	196	166	84.69
12	0	11.98 ± 0.15	42.13 ± 15.48	4	150	5	3.33
12	130	11.99 ± 0.16	157.75 ± 18.70	4	197	1	0.51
		12.00 ± 0.14	157.75 ± 18.70	4	181	0	0.00
12	350	11.89 ± 0.07	388.19 ± 22.20	7	216	210	97.22
		12.00 ± 0.07	386.74 ± 57.94	3	110	107	97.27
12	650	12.00 ± 0.09	615.73 ± 35.43	7	288	281	97.57
		12.08 ± 0.09	618.27 ± 43.36	6	131	125	95.42
17	0	17.18 ± 0.26	53.60 ± 14.30	3	61	2	3.28
		17.12 ± 0.27	53.60 ± 14.30	3	35	2	5.71
17	130	17.22 ± 0.32	157.75 ± 0.00	3	148	2	1.35
		17.20 ± 0.54	157.75 ± 0.00	2	80	5	6.25
17	350	16.90 ± 0.43	376.56 ± 64.67	2	133	78	58.65
		17.02 ± 0.33	390.13 ± 59.75	3	230	214	93.04
17	650	17.01 ± 0.22	636.08 ± 38.55	4	226	196	86.73
		16.97 ± 0.19	647.28 ± 40.55	5	116	108	93.10
20	0	21.48 ± 2.16	40.20 ± 19.44	2	22	0	0.00
		22.25 ± 5.88	41.73	1	5	1	20.00
20	50	21.97 ± 3.53	41.93 ± 10.29	2	4	0	0.00
		22.25 ± 5.88	41.12	1	26	0	0.00
20	230	21.40 ± 2.24	202.91 ± 72.74	2	7	2	28.57
		21.40 ± 2.24	202.91 ± 72.74	2	6	1	16.67
20	490	21.40 ± 2.12	501.24 ± 8.10	2	44	41	93.18
		21.38 ± 1.44	503.78 ± 12.63	3	33	33	100.00

APPENDIX 7B

ANALYSIS OF RAINBOW SMELT CHLORINE/TEMPERATURE BIOASSAY DATA
PERCENTAGE MORTALITY OF NEWLY HATCHED LARVAE

Analysis of Variance Table - Transformed Data

Source	SS	DF	MSS	Approx F	MLR
Regression	5998.2910	5	1199.66	33.56	.311819-14*
Linear	3970.9643	2	1985.48	55.54	.764595-12*
x_1 Linear	74.6108	1	74.61	2.09	.245389+00
x_2 Linear	3783.7418	1	3783.74	105.85	.142462-11*
Quadratic	1361.5392	2	680.77	19.04	.172281-06*
x_1 Quad	28.4933	1	28.49	.80	.575995+00
x_2 Quad	1357.2613	1	1357.26	37.97	.177688-06*
$x_1 * x_2$	.5822	1	.58	.02	.988594+00
Transform	6969.8401	3	2323.28	64.98	.391766-15*
Residual	786.4191	22	35.75		
TOTAL	6784.7101	30			

Relative likelihood of no transformation vs ml estimates = .39177-15

Regression:

$$Y^{.435} = -67.4627 + 91.7743x_1^{.148} + .00000263073x_2^{2.445} - 30.5405x_1^{.296} - (.293659).10^{-12}x_2^{4.8} + .000000161403x_1^{.148}x_2^{2.44}$$

Y = % Mortality of Hatched Larvae

x_1 = Temperature °C

x_2 = Total Residual Chlorine ppb

Mean Residual for Model (31 data points) = 8.9%

APPENDIX 8

TEMPERATURE/CHLORINE BIOASSAY OF RAINBOW SMELT EGGS
DATA FOR TESTS WITH LAKEVIEW GS DILUTION WATER

Experimental Conditions					Results			
Design		Observed ± C.I.			Embryo Survival			
Temp °C	TRC ppb	Temp °C	TRC ppb	Time (Days)	Initial No	Final No	% Survival	% Survival Predicted by Models
BLOCK 1								
17	0	16.85 ± 0.74	3.67 ± 10.35	2	563	333	59.15	40
		15.97 ± 0.87	3.87 ± 18.11	2	494	82	16.60	40
17	350	17.03 ± 0.76	120.10 ± 90.53	2	388	268	69.07	34
		17.03 ± 0.76	120.10 ± 90.53	2	254	75	29.53	34
17	650	17.00 ± 0.79	323.64 ± 168.10	2	607	64	10.54	32
		17.03 ± 0.76	323.64 ± 168.10	2	532	34	6.39	32
BLOCK 2								
17	0	16.48 ± 0.39	1.99 ± 1.44	9	333	255	76.58	72
		16.73 ± 0.30	1.27 ± 1.30	10	82	76	92.68	72
17	350	16.53 ± 0.40	239.28 ± 40.45	9	266	169	63.53	73
		16.60 ± 0.30	239.28 ± 40.45	9	75	52	69.33	73
17	650	16.51 ± 0.46	417.28 ± 46.19	7	64	44	68.75	71
		16.60 ± 0.50	417.28 ± 46.19	7	33	20	60.61	71
TOTAL HATCH								
17	0	16.55 ± 0.32	2.29 ± 1.24	11	563	255	45.29	27
		16.50 ± 0.33	1.79 ± 1.34	12	494	76	15.38	27
17	350	16.62 ± 0.34	217.61 ± 45.31	11	388	169	43.56	24
		16.44 ± 0.35	217.61 ± 45.31	11	254	52	20.47	24
17	650	16.62 ± 0.38	396.47 ± 46.25	9	607	44	7.25	26
		16.69 ± 0.40	396.47 ± 46.25	9	532	20	3.76	26
VIABLE HATCH								
17	0	16.55 ± 0.32	2.29 ± 1.24	11	563	149	26.47	19
		16.50 ± 0.33	1.79 ± 1.34	12	494	54	10.93	19
17	350	16.62 ± 0.34	217.61 ± 45.31	11	388	64	16.49	14
		16.44 ± 0.35	217.61 ± 45.31	11	254	37	14.57	14
17	650	16.62 ± 0.38	396.47 ± 46.25	9	607	18	2.97	7
		16.69 ± 0.40	396.47 ± 46.25	9	532	7	1.32	7
LARVAL DEFORMITY					No Hatched	No Deformed	% Deformed	Predicted
17	0	16.55 ± 0.31	2.29 ± 1.24	11	255	98	38.43	37
		16.50 ± 0.33	1.79 ± 1.34	12	76	18	23.68	37
17	350	16.62 ± 0.34	217.61 ± 45.31	11	169	102	60.35	16
		16.44 ± 0.35	217.61 ± 45.31	11	52	11	21.15	16
17	650	16.62 ± 0.38	396.47 ± 46.25	9	44	2	4.55	10
		16.69 ± 0.40	396.47 ± 46.25	9	20	4	20.00	10
LARVAL MORTALITY					Normal Hatch	Mortalities	% Mortality	Predicted
17	0	16.63 ± 0.66	1.73 ± 3.44	4	157	8	5.10	2
		16.59 ± 0.52	0.51 ± 0.97	4	58	4	6.90	2
17	350	16.75 ± 0.64	273.51 ± 87.54	4	67	3	4.48	20
		16.57 ± 0.47	273.51 ± 87.54	4	41	4	9.76	20
17	650	16.88 ± 1.21	450.38 ± 225.98	2	42	24	57.14	100
		17.15 ± 1.28	450.38 ± 225.98	2	16	9	56.25	100

POOR

QUALITY

ORIGINAL